

AMENDMENTS TO THE CLAIMS

1-69. (Cancelled)

70. (Currently amended) A method for making a purified adenovirus composition comprising:

a) growing host cells in a media;

b) providing nutrients to said host cells by perfusion or through a fed-batch process,
~~fed batch or automated roller bottles;~~

c) infecting said host cells with an adenovirus;

d) lysing said host cells to provide a cell lysate comprising adenovirus; and

e) purifying adenovirus from said lysate by a process other than the use of cesium

chloride density gradient centrifugation to provide a pharmaceutically acceptable purified adenovirus composition having (a) a contaminating nucleic acid content of less than 400 pg per 10^{10} pfu virus and greater than or equal to about 60 pg per 10^{10} pfu virus ~~or (b) a level of BSA that is below the detection level of a Western blot assay.~~

71. (Previously presented) The method of claim 129, wherein the purified adenovirus composition comprises 70% +/- 10% of the starting PFU.

72. (Previously presented) The method of claim 129, wherein the purified adenovirus composition comprises a substantially purified adenovirus composition.

73. (Cancelled)

74. (Previously presented) The method of claim 129, wherein the purified adenovirus composition has a contaminating nucleic acid concentration of less than 0.2 ng/ml.

75. (Previously presented) The method of claim 129, wherein the purified adenovirus composition has an A₂₆₀/A₂₈₀ ratio of between about 1.2 and 1.3.

76. (Previously presented) The method of claim 129, wherein the purified adenovirus composition has an A₂₆₀/A₂₈₀ ratio of 1.27 +/- 0.03.

77. (Previously presented) The method of claim 129, wherein the purified adenovirus composition has a contaminating nucleic acid concentration of less than 0.8 ng/ml and an A₂₆₀/A₂₈₀ ratio of between about 1.2 and 1.3.

78. (Canceled).

79. (Previously presented) The method of claim 129, wherein the media is serum-free.

80. (Previously presented) The method of claim 129, wherein the host cells are grown in a bioreactor.

81. (Previously presented) The method of claim 129, wherein the host cells are grown on microcarriers.

82. (Previously presented) The method of claim 129, wherein said purified adenovirus composition comprises an adenoviral vector encoding an exogenous gene construct.

83. (Previously presented) The method of claim 82, wherein said exogenous gene construct is operatively linked to a promoter.

84. (Previously presented) The method of claim 83, wherein said promoter is SV40 IE, RSV LTR, β -actin, CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.

85. (Previously presented) The method of claim 82, wherein said exogenous gene construct encodes a therapeutic gene.

86. (Previously presented) The method of claim 85, wherein said therapeutic gene encodes antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense *jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl*, antisense *abl*, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, zac1, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-1, MEN II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2,

IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, GM-CSF, G-CSF, thymidine kinase or p53.

87. (Previously presented) The method of claim 86, wherein said therapeutic gene encodes p53.

88. (Previously presented) The method of claim 129, wherein the adenovirus in said purified adenovirus composition is a replication-incompetent adenovirus.

89. (Previously presented) The method of claim 88, wherein the adenovirus in said purified adenovirus composition is lacking at least a portion of the E1-region.

90. (Previously presented) The method of claim 89, wherein the adenovirus in said purified adenovirus composition is lacking at least a portion of the E1A and/or E1B region.

91. (Previously presented) The method of claim 88, wherein said host cells are capable of complementing replication.

92. (Previously presented) The method of claim 129, wherein said host cells are 293 cells.

93. (Previously presented) The method of claim 129, wherein said lysate is treated with a nuclease.

94. (Previously presented) The method of claim 129, wherein said purified adenovirus composition comprises a pharmaceutically acceptable buffer.

95. (Previously presented) The method of claim 94, wherein said purified adenovirus composition provides a unit dose of between 10^3 and 10^{15} PFU/dose.

96. (Previously presented) The method of claim 94, wherein the purified adenovirus composition provides a unit dose of between 10^{10} and 10^{14} PFU/dose.

97. (Previously presented) The method of claim 129, wherein said purifying is characterized by a process that includes at least one chromatography step to provide a pharmaceutically acceptable composition.

98. (Previously presented) The method of claim 129, wherein said purifying is characterized by a process that includes a single chromatography step to provide a pharmaceutically acceptable composition.

99. (Withdrawn) The method for making a purified adenovirus composition for therapeutic use comprising:

- a) growing host cells in a media comprising glucose;
- b) infecting said host cells with an adenovirus;
- c) lysing said host cells by a lysis method other than freeze-thaw to produce a lysate comprising said adenovirus composition; and

d) purifying adenovirus from said lysate to provide a purified adenovirus composition for therapeutic use.

100. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use comprises 70% +/- 10% of the starting PFU.

101. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use comprises a substantially purified therapeutic adenovirus composition.

102. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use has a contaminating nucleic acid concentration of less than 0.8 ng/ml.

103. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use has a contaminating nucleic acid concentration of less than 0.2 ng/ml.

104. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use has an A₂₆₀/A₂₈₀ ratio of between about 1.2 and 1.3.

105. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use has an A₂₆₀/A₂₈₀ ratio of 1.27 +/- 0.03.

106. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use has a contaminating nucleic acid concentration of less than 0.8 ng/ml and an A₂₆₀/A₂₈₀ ratio of between about 1.2 and 1.3.

107. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use has a BSA content below the detection level of a western blot assay.

108. (Withdrawn) The method of claim 99, wherein the media is serum-free.

109. (Withdrawn) The method of claim 99, wherein the host cells are grown in a bioreactor.

110. (Withdrawn) The method of claim 99, wherein the host cells are grown on microcarriers.

111. (Withdrawn) The method of claim 99, wherein said purified adenovirus composition for therapeutic use comprises an adenoviral vector encoding an exogenous gene construct.

112. (Withdrawn) The method of claim 111, wherein said exogenous gene construct is operatively linked to a promoter.

113. (Withdrawn) The method of claim 112, wherein said promoter is SV40 IE, RSV LTR, β -actin, CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.

114. (Withdrawn) The method of claim 111, wherein said exogenous gene construct encodes a therapeutic gene.

115. (Withdrawn) The method of claim 114, wherein said therapeutic gene encodes antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense *jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl*, antisense *abl*, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, zac1, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-1, MEN II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, GM-CSF, G-CSF, thymidine kinase or p53.

116. (Withdrawn) The method of claim 115, wherein said therapeutic gene encodes p53.

117. (Withdrawn) The method of claim 99, wherein said purified adenovirus composition for therapeutic use is a replication-incompetent adenovirus.

118. (Withdrawn) The method of claim 117, wherein said purified adenovirus composition for therapeutic use is lacking at least a portion of the E1-region.

119. (Withdrawn) The method of claim 118, wherein the purified adenovirus composition for therapeutic use is lacking at least a portion of the E1A and/or E1B region.

120. (Withdrawn) The method of claim 99, wherein said host cells are capable of complementing replication.

121. (Withdrawn) The method of claim 99, wherein said host cells are 293 cells.

122. (Withdrawn) The method of claim 99, wherein said lysate is treated with a nuclease.

123. (Withdrawn) The method of claim 99, wherein said purified adenovirus composition for therapeutic use comprises a pharmaceutically acceptable buffer.

124. (Withdrawn) The method of claim 123, wherein said purified adenovirus composition for therapeutic use provides a unit dose of between 10^3 and 10^{15} PFU/dose.

125. (Withdrawn) The method of claim 123, wherein the purified adenovirus composition for therapeutic use provides a unit dose of between 10^{10} and 10^{14} PFU/dose.

126. (Withdrawn) The method of claim 99, wherein said purifying is characterized by a process that includes at least one chromatography step capable of providing a purified therapeutic adenovirus composition.

127. (Withdrawn) The method of claim 99, wherein said purifying is characterized by a process that includes a single chromatography step capable of providing a purified therapeutic adenovirus composition.

128. (Withdrawn) The method of claim 99, wherein the lysis method other than freeze-thaw is a process that includes hypotonic solution, hypertonic solution, impinging jet, microfluidization, solid shear, detergent, liquid shear, high pressure extrusion, autolysis or sonication to produce a crude lysate composition comprising adenovirus.

129. (Canceled)

130. (Previously presented) The method of claim 129 wherein the purified adenovirus composition has a contaminating nucleic acid concentration of less than 0.8 ng/ml.

131. (Previously presented) The method of claim 129 wherein the purified adenovirus composition has a contaminating nucleic acid content of less than about 140 pg per 10^{10} pfu virus and greater than or equal to about 60 pg per 10^{10} pfu virus.

132. (Previously presented) The method of claim 129 wherein the particle to pfu ratio is between about 36 and about 38.

133. (Previously presented) The method of claim 70 wherein step e) comprises purifying adenovirus from said lysate by a process other than the use of cesium chloride density gradient centrifugation to provide pharmaceutically acceptable purified adenovirus composition having (b) a level of BSA that is below the detection level of a Western blot assay.

134. (Previously presented) The method of claim 133, wherein the purified adenovirus composition comprises 70% +/- 10% of the starting PFU.

135. (Previously presented) The method of claim 133, wherein the purified adenovirus composition comprises a substantially purified adenovirus composition.

136. (Previously presented) The method of claim 133, wherein the purified adenovirus composition has a contaminating nucleic acid concentration of less than 0.2 ng/ml.

137. (Previously presented) The method of claim 133, wherein the purified adenovirus composition has an A₂₆₀/A₂₈₀ ratio of between about 1.2 and 1.3.

138. (Previously presented) The method of claim 133, wherein the purified adenovirus composition has an A₂₆₀/A₂₈₀ ratio of 1.27 +/- 0.03.

139. (Previously presented) The method of claim 133, wherein the purified adenovirus composition has a contaminating nucleic acid concentration of less than 0.8 ng/ml and an A₂₆₀/A₂₈₀ ratio of between about 1.2 and 1.3.

140. (Previously presented) The method of claim 133, wherein the media is serum-free.

141. (Previously presented) The method of claim 133, wherein the host cells are grown in a bioreactor.

142. (Previously presented) The method of claim 133, wherein the host cells are grown on microcarriers.

143. (Previously presented) The method of claim 133, wherein said purified adenovirus composition comprises an adenoviral vector encoding an exogenous gene construct.

144. (Previously presented) The method of claim 143, wherein said exogenous gene construct is operatively linked to a promoter.

145. (Previously presented) The method of claim 144, wherein said promoter is SV40 IE, RSV LTR, β -actin, CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.

146. (Previously presented) The method of claim 143, wherein said exogenous gene construct encodes a therapeutic gene.

147. (Previously presented) The method of claim 146, wherein said therapeutic gene encodes antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense *jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl*, antisense *abl*, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, zac1, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-1, MEN II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, GM-CSF, G-CSF, thymidine kinase or p53.

148. (Previously presented) The method of claim 147, wherein said therapeutic gene encodes p53.

149. (Previously presented) The method of claim 133, wherein the adenovirus in said purified adenovirus composition is a replication-incompetent adenovirus.

150. (Previously presented) The method of claim 149, wherein the adenovirus in said purified adenovirus composition is lacking at least a portion of the E1-region.

151. (Previously presented) The method of claim 150, wherein the adenovirus in said purified adenovirus composition is lacking at least a portion of the E1A and/or E1B region.

152. (Previously presented) The method of claim 149, wherein said host cells are capable of complementing replication.

153. (Previously presented) The method of claim 133, wherein said host cells are 293 cells.

154. (Previously presented) The method of claim 133, wherein said lysate is treated with a nuclease.

155. (Previously presented) The method of claim 133, wherein said purified adenovirus composition comprises a pharmaceutically acceptable buffer.

156. (Previously presented) The method of claim 155, wherein said purified adenovirus composition provides a unit dose of between 10^3 and 10^{15} PFU/dose.

157. (Previously presented) The method of claim 155, wherein the purified adenovirus composition provides a unit dose of between 10^{10} and 10^{14} PFU/dose.

158. (Previously presented) The method of claim 133, wherein said purifying is characterized by a process that includes at least one chromatography step to provide a pharmaceutically acceptable composition.

159. (Previously presented) The method of claim 133, wherein said purifying is characterized by a process that includes a single chromatography step to provide a pharmaceutically acceptable composition.

160. (Previously presented) The method of claim 133 wherein the purified adenovirus composition has a contaminating nucleic acid concentration of less than 0.8 ng/ml.

161. (Previously presented) The method of claim 133 wherein the purified adenovirus composition has a contaminating nucleic acid content of less than about 140 pg per 10^{10} pfu virus and greater than or equal to about 60 pg per 10^{10} pfu virus.

162. (Previously presented) The method of claim 133 wherein the particle to pfu ratio is between about 36 and about 38.